Application No. 09/920,435
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REMARKS

Claims 1-14 and 21-22 are pending in the present application and claims 15-20 were withdrawn from consideration due to a restriction requirement. Claims 15-20 are cancelled without prejudice herein. Claim 1 is amended herein. Accordingly, claims 1-14, 21 and 22 will be pending upon entry of the instant amendments.

Support for the amended claim can be found throughout the specification and encompassed by the scope of the claims as originally filed. For example, support for the amendment to claim 1 can be found, at least, for example, on page 1, lines 17-20. No new matter has been added.

Any amendments to the claims should in no way be construed as acquiescence to any of the Examiner's rejections and were done solely to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

Claim Rejections - 35 U.S.C. §112

Claims 1-14 and 21-22 remain rejected under 35 U.S.C. §112, second paragraph, as being indefinite for the term "natural." The Examiner declares that "[a]bsent of a teaching of ***all*** naturally occurring samples, one would not be able to determine which were or were not made by a natural process because both the "natural"

and "unnatural" samples would have the same structures i.e., you couldn't tell just by looking at them."

Applicants respectfully traverse the foregoing rejection.

However, Applicants amended claim 1 to change the term "natural" to a "complex biological material". A sample of complex biological material, in accordance with the invention, is a crude extract or a purified fraction of material derived from plants, animals, microorganisms, or other biological sources, which contains a complex mixture of compounds of natural origin. Such samples may contain a wide variety of compounds, which span a wide range of molecular weights. These samples may contain small inorganic or organic species (e.g., < 1000 Da); small to moderate size organic compounds (e.g., < 5000 Da); oligomeric biomolecules such as oligosaccharides, peptides, oligonucleotides, and lipids (e.g., < 5000 Da); polymeric biomolecules such as proteins, polynucleotides, and polysaccharides (e.g., 5-500 kDa); and/or large macromolecular structures (e.g., > 500 kDa). (*See*, page 8, lines 6-32, and page 11, lines 25-32, of the specification.)

What makes the present sample in the present method distinguishable from other samples to be tested is that the present method requires an additional step of passing the reaction mixture through a second size exclusion medium that removes from the reaction mixture any molecule larger than a second preset value. Synthetic samples, e.g., combinatorial libraries, do not require this extra step. Moreover, structural features of samples are not discernable unless they are fully analyzed. In

combination with this additional step, the recitation of "a sample of complex biological material" or even a "natural" sample is appropriate and clear to one of ordinary person skilled in the art. Accordingly, claim 1 and its subsequent dependent claims 2-14 and 21-22 should now be clear and definite. Applicants respectfully request reconsideration and withdrawal of the foregoing rejection.

Claim Rejections – 35 U.S.C. §102

Claims 1-14 and 21-22 remain rejected under 35 U.S.C. §102(e) as being anticipated by Nash et al. (U.S. Patent 6,207,861).

Applicants respectfully traverse the foregoing rejection.

The present invention is unique in that the screening methodology is primarily for complex biological material samples, as described above, which contain small to large molecules with different complexities. Additionally, the method of screening requires an additional step of passing the reaction mixture through a second size exclusion medium that removes from the reaction mixture any molecule larger than a second preset value. Natural extracts often contain molecules with molecular weights equal to or larger than the target being screened, and in the first filtration step, these molecules will co-elute from the size-exclusion medium along with the target/hit complex. Injecting these large molecules into a reverse-phase HPLC system often results in fouling and clogging of the column and transfer lines, damaging the system. Therefore, the present invention has

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discovered that a second size separation, such as passing the sample through an ultrafiltration membrane under dissociating conditions, is needed to filter out all of the large molecules, including the target as well. This additional step would then provide the ability to isolate and analyze the small molecule that had genuine affinity for the target.

It is well established that for a prior art reference to anticipate a claimed invention under 35 U.S.C. §102, the prior art must teach each and every element of the claimed invention. Nash et al. fails to anticipate each and every element of the claimed invention. As previously argued, Nash et al. is directed to a method of producing a mass-coded set of synthetic chemical compounds to be used as a screening tool for identifying a ligand which binds to a particular biomolecule. Nash et al. focuses on screening combinatorial, small molecule libraries rather than natural samples. Nash et al. applies a single filtering step that removes unbound small molecules, such as, using a gel filtration column. Therefore, Nash et al. was intent only on separating out unbound, small ligands from larger complexes of small ligands bound to target, which were then subjected to mass spectrometry.

Nash et al. makes no reference to using their screening method with samples of complex biological material, such as natural extracts. While the Examiner recites that Nash et al. describes the sample to be a biomolecule that can be a protein, it only provides a generalized statement that the biomolecule can be a protein or a nucleic acid molecule, such as a DNA or RNA. This neither anticipates nor suggests and teaches anything with regard

to a screening method of a complex biological material sample. Nash et al. produces synthetic libraries of mass-encoded mixtures and uses its method to screen them. In Nash et al., any small molecule bound to the target of interest passes through the size-exclusion column, and this complex is directed to an HPLC column, where the separation process causes dissociation of the complex and release of the active compound. Because the mixture of compounds used in Nash is carefully pre-engineered so that every mass is unique, the mass spectrometer gives an immediate answer as to which synthetic compound was bound to the target.

The method employed in Nash et al. cannot be directly used nor would it work for screening a complex biological mixture of natural origin. Because natural extracts often contain molecules with molecular weights equal to or larger than the target being screened, these molecules will elute from the size-exclusion medium along with the target/hit complex. If a mixture of target and the complex biological material sample was analyzed using the procedure described by Nash et al., one of ordinary person skilled in the art would not be able to determine the species that was bound to the target; in the final LC-MS analysis, a mixture would be observed and not a clearly isolated compound. Furthermore, as mentioned above, injecting these large molecules into reverse-phase HPLC often results in clogging of the column and transfer lines.

Nash et al. fails to anticipate using a second size-separation medium for removing any molecules larger than a second preset value. While the Examiner argues that "Nash

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discloses subjecting the reaction mixture to a second size exclusion medium," it is unclear how this is so when Nash et al.'s method does not require such a step based on the combinatorial library used and when Nash et al. plainly fails to teach or suggest, or provide any requisite motivation, to using a second size exclusion medium. Applicants consider that Nash et al. fails to anticipate each and every element of the claimed invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of the foregoing rejection.

Claim Rejections - 35 U.S.C. §103

Claims 1-14 and 21-22 remain rejected under 35 U.S.C. §103(a) as being obvious over Kaur et al. (*J Prot Chem* 1997, 16(5):505-511) in view of Van Breeman et al. (*Anal Chem* 1997, 69:2156-2164).

Applicants respectfully traverse the rejection.

Similar to Nash et al., Kaur et al. also uses a method of identifying ligands by screening libraries of small molecules created by combinatorial synthetic methods. Kaur et al. is distinguishable from the present invention for, again, the following reasons. The present method requires a second filtration step to remove large molecules under dissociating conditions specifically for the purpose of analyzing complex biological samples. In addition, samples of complex biological material, such as natural extracts, cannot be used in the method taught in Kaur et al. since it only allows binding of compounds

with the highest affinities to bind to the receptor by using receptor-limiting incubation conditions. Kaur et al. fails to teach or suggest, or fails to provide any requisite motivation, to come up with a method for screening complex biological material samples and it also fails to teach or suggest using a second size exclusion medium.

Van Breemen et al. fails to cure the deficiencies found in Kaur et al. It seems the Examiner is making the connection that Van Breemen et al. teaches or suggests substituting an ultrafiltration membrane as a second size-exclusion medium in combination with the LC-MS portion of the hyphenated SEC-LC-MS method as taught by Kaur et al. "(i.e., a hyphenated SEC-ultrafiltration-MS method would result after substitution)," because Van Breemen et al. explicitly states that "[a] liquid chromatograph-electrospray mass spectrometer (LC-MS) was used as a screening apparatus, except that an ultrafiltration chamber was substituted for the HPLC column."

Contrary to the Examiner's assertion, Van Breemen et al. fails to teach or suggest that an ultrafiltration-mass spectrometry can be used as a substitute for a liquid chromatography-mass spectrometry. This is a subtle misinterpretation of the language in the Experiment Section of the cited publication. A "liquid chromatograph-electrospray mass spectrometer" is an instrument; "liquid chromatography-electrospray mass spectrometry" is a technique. For example, if a liquid chromatograph-electrospray mass spectrometer is used and an HPLC column is not installed, then only mass spectrometry

is being performed, using the pumping and valving system of the chromatograph to deliver unseparated samples. Similarly, if one installs an ultrafiltration chamber inside the liquid chromatograph, ultrafiltration-MS would be performed. Thus, Van Breemen et al. did not teach one to substitute ultrafiltration-MS technique for LC-MS technique; all that is implied is that an ultrafiltration chamber can be installed inside a liquid chromatograph instrument. Therefore, no teaching or suggestion can be gleaned from the methods employed in Van Breemen et al., either alone or in combination with Kaur et al., to come up with the present invention. Van Breemen et al. fails to teach or suggest providing a second size exclusion medium for removing large molecules regardless of what method is used as the medium.

Moreover, an ordinary skilled artisan would not find it obvious to even combine the teachings of both Kaur et al. and Van Breemen et al. When screening against mixtures of synthetic drug-like compounds, which typically are only small molecules (less than 2000 Da), both methods used in the cited publications would be similarly effective. All non-binding small molecule synthetic compounds are removed by either technique alone, so that an ordinary skilled artisan would not even make the suggested combination. However, with the combined method steps claimed in the present invention, samples of complex biological material, such as natural extracts, can be analyzed efficiently by first using a size-exclusion step to remove the small molecule non-binders, and then a second filtration step to remove the large molecule non-binders. This is plainly not taught nor suggested in either Kaur et al. or Van Breemen et

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al., alone or in combination, to make the present invention obvious. Applicants respectfully request reconsideration and withdrawal of the foregoing rejection.

Based on the foregoing, entry of the amendments and remarks presented herein, reconsideration and withdrawal of all the rejections and allowance of application with all pending claims are respectfully requested.

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The Examiner is encouraged to telephone the undersigned attorney to discuss any matter that would expedite allowance of the present application.

Respectfully submitted,

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